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THE JOHNS HOPKINS PRESS

BALTIMORE, MARYLAND, U. S. A.

A CASE OF PROGRESSIVE NEURAL MUSCULAR ATROPHY

SAMUEL JOSEPH WEINBERG

From the Medical Clinic of the Johns Hopkins Hospital

In 1886 Charcot and Pierre Marie (2) described a new disease entity which they defined as a "peculiar form of progressive muscular atrophy." This type of muscular atrophy has been frequently subdivided. The majority of cases recorded up to the present time still fall within the bounds of the original description, but we know no more concerning the mechanism of its causation than did Charcot and Marie when they described their 5 cases. Tooth (26), whose name is generally linked with those of Charcot and Marie, in his doctoral thesis at Cambridge (1886) attempted independently to show the distinctiveness of this condition, describing it as the "peroneal type of progressive muscular atrophy."

In the words of Charcot and Marie (2)

". . . it follows that the principal characters of the form of muscular atrophy which we propose to isolate and describe are the following:

"Progressive muscular atrophy attacking first the feet and legs but not appearing on the upper extremities (hands first, then forearms) until several years later; hence, a slow progression.

"Relative integrity of the muscles of the limbs near the trunk, or at least longer preservation of these than of the muscles at the distal ends of the extremities.

"Integrity of the muscles of the trunk, shoulders and of the face. Existence of fibrillary contractions in the muscles undergoing atrophy. Vasomotor disturbances in the parts of the limbs atrophied. No pronounced contractions of tendons in the atrophied limbs. Sensation most often intact but sometimes altered in various ways. Frequency of cramps and reactions of degeneration in the atrophic muscles. The affection begins usually in childhood, often in several brothers and sisters, and sometimes exists also in the previous generation."

To this list of symptoms Tooth (27) adds the following modifications:

"The disease shows a slight preference for the male sex. Fibrillar or fascicular tremors are frequently though not always present, and degenerative electrical reactions often appear early."

FIG. 1

CLINICAL HISTORY

The patient B. C., aged twenty, a native of Western Maryland, was admitted to the hospital March 16, 1931 (Unit No. 36082), complaining of discharging sores, which he had had on the bony prominences of his extremities for eighteen

years, and progressive muscular wasting and extreme deformity of the extremities of ten years' duration. His father, mother, two sisters, and a maternal uncle are in excellent health. A paternal aunt has a diffusely enlarged goitre and evi-

FIG. 2

dences of hyperthyroidism. In none of the immediate family is there any evidence of a condition simulating that of the patient. There is an indefinite history of a similar condition in a paternal uncle, now dead. The mother, however, insists that the youngest boy of the elder sister of the patient, aged two years and nine

months, has an affection of the lower extremities identical with that seen in our patient at the onset of his malady at the age of two. This will be described below. There is no history of other familial disease.

Except for the milder diseases of childhood and the present illness, the patient has always been in good health and very active within the limits imposed by his deformities. His *present illness* appears to have begun at the age of two with areas of cyanosis and sores at various places on the feet and shortly thereafter on the hands. His condition remained essentially the same for several years and during that time he was much more sensitive to coldness of the lower extremities than were his sisters. At the age of ten it was first noticed that there was wasting of the muscles of the legs involving chiefly those below the knees, and from that time to the present the atrophy and deformity have made more rapid progress. He states that his condition in regard to the muscular atrophy was far advanced at the age of ten, but that the contractions of the wrists have come on since and so has also the "drawing up" of the feet. Five years ago he could walk barefoot, but since then a specially made canvas shoe has been necessary. Sores appearing and healing independently, leaving dense indurated scars, were always present on the bony projections of the extremities. For a few years, beginning at the age of ten, similar sores occurred in aggravated form over the soft parts of the extremities, followed by scars. No such sores were situated on the trunk, but some appeared from time to time on the margins of the ears and one a year ago on the scrotum. In the last two years mottled cyanotic areas of discoloration have appeared from time to time on the face just below the eyes. He has always been active and out of doors and until the past year was able to walk four to five miles daily. There is no history whatsoever of arthritis. He has never experienced cramps, lightning pains, or any difficulty in locomotion aside from that caused by pressure on the sores on the feet while walking. No paralyses, tremors, nystagmus or ataxia have ever been noted.

Condition on admission. At present the range of his activities, considering the extreme muscular atrophy, is truly remarkable. He is able to dress and feed himself, can raise a liter pitcher of water to fill a glass, writes, and when at home can swing a scythe or help to repair an automobile. Of late he has noticed that he tires more easily on effort.

On examination the patient is fairly well nourished and certainly of good intelligence. The extent of his muscular atrophy and the extreme contractions are well shown in the photographs (figs. 1 and 2). Over the bridge of the nose, on the ears, and on the hands and feet the skin is mottled, thin, tightly drawn and cyanotic. Crusts are present on the bony projections of the feet, hands, and at the elbows. None of the stigmata of congenital syphilis and no evidence of rickets are to be seen.

The *head* is normally formed. There is no tenderness over the skull or the sinuses. There are no exostoses. The lower portion of the bony bridge of the nose is irregular and roughened, but there is no suggestion of a saddle-back nose. The septum is intact. A few fine hairs are seen over the face and in the axillae. The skin of the face, arms and hands has a distinctly cyanotic color.

Eyes. The puffiness and bluish discoloration about and chiefly below the eyes have been noted. The palpebral fissures are of normal width. There is no exophthalmos. Conjunctivae not injected. Sclerae of normal color. The pupils are round, symmetrical and react to light and accommodation; the extra-ocular movements are well performed. Ophthalmoscopic examination reveals no abnormality.

Ears. The helix on the left is almost obliterated by what appears to have been an old ulceration now completely healed. The right helix is less impaired. Hearing is unimpaired.

The *teeth* are in extremely poor condition. The upper front teeth are carious stumps. The remaining teeth are in a wretched state with many cavities. There is, however, no suggestion of pegging or of extra cusps of the intact teeth.

The *tonsils* are small and unscarred, the pharynx clear. The glands of the neck are moderately enlarged, but there is no general glandular enlargement.

The right side of the thorax is slightly more prominent than the left. The *lungs* are normally resonant throughout. No râles are heard and the bases descend well. There is no hair on the chest. The cardio-vascular system shows no abnormality. The vessel walls are not thickened. The *heart* is not enlarged. The heart sounds are of good quality and perfectly clear. No murmurs present. The blood pressure is 115/75. The heart rate 78.

The *abdomen* is soft and flat. No tenderness or muscle spasm was noted. The liver dulness extends just below the costal edge in the mid-clavicular line. With the patient turned on the right side the spleen descends one and a half fingers' breadth below the costal margin. There is no hair upon the abdomen. The pubic hair is fairly heavy but has a transverse feminine distribution.

The *genitalia* are normal. The scrotal sac over its lower half is moderately swollen and of a dull red color. There are several scars of small ulcers over the lower scrotum. On palpation, in this lower part of the scrotum a more tender doughy subcutaneous mass is felt, apparently the result of old inflammation.

Extremities. The extremities show the most extreme degree of atrophy, with contractures and the resulting deformities. The interference with the normal mechanical function has been excessive and yet has in large measure been overcome by the adaptation of the individual to the long-standing duration of the disease. The upper extremities are of very small circumference. The measurements are as follows:

	CIRCUMFERENCE OF LEFT UPPER ARM	CIRCUMFERENCE OF RIGHT UPPER ARM
	cm.	*cm.*
Above wrist	7.3	8.5
Mid-forearm	10	9.5
Below elbow	12.5	13.1
Above elbow	13	13
Mid-arm	13.2	12.5
Below shoulder	13.5	14.6

There are flexor contractures of the elbows and wrists. The elbows can not be extended beyond 30°, while the wrists are so over-flexed that the hands lie against the anterior forearms. The hands are small, the fingers are extended at the metacarpo-phalangeal joints and flexed at a fixed angle of 90° at the first phalangeal joints. There is the barest amount of motion in these joints. The bony prominences on the extensor surfaces of these permanently flexed joints are very prominent and covered with thin shiny white skin which is ulcerated over the olecranons. The shoulders have limited motion (the left one being almost completely ankylosed), most of the motion coming from the scapulae.

The hips are normal. The characteristic spindle shape of the legs is well shown in the accompanying photograph. There is slight atrophy of the thighs. The knees have a fairly normal range of movement; complete extension is difficult. The patellae and condyles are prominent because of soft tissue atrophy.

The lower legs are of very small circumference. Measurements are as follows.

	LEFT LOWER EXTREMITY	RIGHT LOWER EXTREMITY
	cm.	*cm.*
Ankle (just above malleoli)	19.5	19.5
Mid-leg	15	14.7
Below knee (lower edge of tibial tuberosity)	20.5	21.9
Above knee (upper edge of patella)	24.5	25
Mid-thigh	33	35
Below hip	37	38

There are scars of recent ulcers on the shins and feet. There is an open ulcer, 2 cm. in diameter, over the left tendo achillis. There is very limited motion of the ankles. The feet tend to assume an equino-varus position. There is extensive atrophy of the small muscles of the feet, as of those of the lower leg.

The toes are greatly distorted, the great toes lying transversely across in front of the ball of the foot and under the second and third toes. The fourth and fifth toes are bent medially. The feet are flattened out and the patient walks chiefly on the calcanei. There is considerable rotation inwards of the left foot.

The *neuro-muscular apparatus* is of especial interest. The patient is quick and alert, with neither intention tremor nor ataxic movements. The biceps muscles are surprisingly strong, considering their size. Flexion at the wrist is strong, extension weak. There is no demonstrable abnormality of function of the cranial nerves and the musculature of the neck and trunk is of unimpaired strength. As noted above, there is partial ankylosis at the wrist and ankle. The periosteal-radial and patellar reflexes are equal and active, the biceps responses are doubtful owing possibly to mechanical interference. The peripheral nerves are not palpable, nor is there any sensitivity to pressure over their course. Fibrillary twitchings are at no time to be observed over the atrophied muscles. There is no diminution in perception of touch, pain, heat, cold or vibrations.

Deep muscle sensibility is unimpaired. Electrical examination of even the smaller muscles shows that there is response to both faradic and galvanic current of intensity within the limits employed for stimulation of normal musculature. There are no reactions of degeneration.

Special examinations. The blood picture is essentially normal except for a lymphocytosis of 41 per cent. R.B.C., 4,120,000. W.B.C., 6,400. Haemoglobin, 72 per cent. Differential count, PM. N. 50 per cent. L.L., 18. S.L., 23. Monos, 6. P.M.B., 1. P.M.E., 2. Platelets, 240,000. Bleeding and clotting time not prolonged. Erythrocyte fragility test normal. Urine. Sp. gr. 1017. Acid. No albumin or sugar. Microscopical examination negative. Examination of stools negative. Temperature 98.6.° Not elevated at any time. Blood chemistry: N.P.N. 32 mg. per cent. Uric acid 3.6 mg. per cent. Calcium 9.69 mg. per cent. Phosphorus 3.9 mg. per cent. Wassermann negative. Repeated smears of nasal secretions and material from ulcers stained with carbolfuchsin reveal no acid-fast bacilli. Cultures made from the ulcers showed only hemolytic staphylococci.

X-ray findings (see fig. 3). All the bones of the extremities are thin and delicate. The distal phalanges of the feet have lost their normal spade-like form and have acquired the characteristic spear-like shape seen in neural lesions. There is no periosteal involvement. In the chest plate the ribs are horizontal, quite widely spaced, and the thorax is of the box-like or emphysematous type; the ribs even show conspicuous thinning. A cervical left-sided scoliosis of moderate degree is evident. The deformities from the contractures, and in the feet from the added pressure effects, are conspicuous.

Reference has been made to a nephew of the patient, two years and nine months old, who is said to have a condition of his feet and legs identical with that which was present at the onset of the illness in the case we are reporting. It was learned that this child was at the Children's Hospital School in Baltimore. Dr. McEachern, under whose direct care the patient came and to whose clinical notes we are greatly indebted, was good enough to see this child and made the following note:

"This child, aged two years and nine months, nephew of the patient B. C., was slightly undersized, but well nourished and showed no deformity whatever. The skin generally, except as noted below, and the mucous membranes were normal. The fontanelles were closed and there was no evidence of rickets elsewhere. The eyes and ears appeared normal. The teeth were well formed and normal. The throat was normal. The heart and lungs were normal, the radial pulses equal and regular. The upper extremities were perfectly normal. The spleen and liver were not palpable. Our interest was centered upon the feet which showed a truly remarkable condition.

FIG. 3

"The four small toes of the left foot and the great toe, and the first and fourth toes of the right foot were cyanosed, some more markedly than others. The capillary response to pressure in these digits, before color returned, was four or five times longer than normal. On the second toe of the left foot the change involved the proximal phalanges but the most distal one was of good color. Similarly, along the borders of both feet the color was poor, but the response to pressure active. A number of reddish or red-blue areas about the size of a ten cent piece were observed along the margins of the feet, and over them the skin was a little thickened. A few similar areas were found over the soles, but here the skin was thicker and the areas were a little suggestive of early trophic ulcers. None of these areas were tender, but both feet were quite cool. The dorsalis pedis pulsations were felt on both sides. No change in color of the patches occurred when the limbs were elevated."

Dr. McEachern later went to see the patient's family in Rawlings, Maryland. He was told by the mother of our patient, grandmother of the nephew referred to above, that the condition of the latter's feet was in every way identical with the early changes that had appeared in her son, the subject of this report, at the onset of his illness at the same age. There is, then, fairly satisfactory evidence supporting the inference that the nephew's vascular changes are early symptoms of the development of a trophic disturbance similar to that of our patient B. C. and that we are dealing with two instances of progressive neural muscular atrophy occurring in the same family, illustrating the very early and the very late symptomatology. It is, of course, understood that the positive diagnosis in the case of the nephew can not be made at this time.

DISCUSSION

It will be seen that the case we are reporting falls well within the bounds set by the authors mentioned above in delimiting progressive neural muscular atrophy, but that in certain respects, notably the absence of fibrillary muscle contractions, cramps, and in the electrical reactions, it does not, and it is on such variants and their clinical emphasis that subdivisions have been made possible.

It may be of value to recount briefly the steps leading to our present knowledge of the condition, touching on points of interest in connection with this case and bearing in mind that the affection is wholly distinct from the myopathies, on the one hand, such as:

1. The facio-scapulo-humeral atrophy (Landouzy-Déjerine: Duchenne's infantile) its variant,

2. Erb's scapular atrophy (Erb's juvenile form) and

3. Pseudo-hypertrophic muscular dystrophy, and from the myelopathies on the other as:

1. Progressive spinal muscular atrophy of infants (Werding-Hoffmann, Oppenheim).

2. Progressive spinal muscular atrophy of adults (Duchenne-Aran; Duchenne's myelopathy).

It may in a sense be said to occupy an intermediate position.

Following the announcements of Charcot-Marie (2) and Tooth (26) great interest was aroused. J. Hoffmann (13, 14), writing in 1888 and again in 1890 from Erb's Clinic in Heidelberg, was quick to search the earlier literature for cases to fit the category, and added to them several observations of his own. It is of interest to note that Friederich Schultze (23), of Bonn, whose observations of 1884 were taken up by Hoffmann, has recently (1930) written regarding the earlier descriptions. In his first work Hoffmann (13), having found autopsies by Virchow (30) (1885), and Friedreich (8) (1873), and having noted changes in the nerves not characteristic of a neuritis, decided to call the condition "neurotic." In 1890 he changed the name to "neural" the term that is used at present.

Virchow described autopsy findings in the case of a man of forty-four in whom the atrophy began at the age of twenty-one first in the lower extremities and then in the upper (both lower and upper arm), and who before death was entirely paralyzed. There is a history of heredity, in that the father before death was in the same state, the atrophy having begun in his case at the age of forty. At autopsy the spinal cord and nerve roots appeared normal but on section there was found gray degeneration in the posterior columns, especially in the columns of Goll, with disappearance of nerve fibers. "The nerves appear to have fewer fibers than normal since on both longitudinal and cross-section there appear wide spaces between the fibers which are filled by a highly nucleated tissue. . . . Microscopic section showed that only the posterior columns were affected, the posterior horns not participating in the degeneration. . . . To the naked eye the nerves were in no wise atrophied and several, for instance, the N. tibialis posticus, even appeared somewhat thick." Similarly, the second case discussed by Friedreich in his noteworthy volume on muscular atrophy (8) (1873), was that of a woman, aged thirty-seven, whose legs began to atrophy at ten, and the arms half a year later with almost total atrophy in a few years. There was severe pain with fibril-

lary twitchings of the muscles of the extremities. The case is singular in that the legs, though remaining weak so that the woman was bed-ridden for years, regained their volume and appeared normal in size at autopsy. At autopsy nothing was found in brain or meninges. On section of the spinal cord there was found gray degeneration of the columns of Goll with characteristic microscopic picture. Both anterior and posterior roots were altered. Few nerve fibers were normal. Many showed varicosities and myelin degeneration; of some only the empty sheaths remained. Everywhere between nerve fibers and fiber bundles was a very rich fibrillar connective tissue in which appeared many long nuclei. The posterior roots of lumbar and sacral segments showed the same changes to lesser degree while the roots of the upper cord were normal. The peripheral nerves were also degenerated and the process continued to the smallest intermuscular nerve twigs where the disappearance of nerve fibers was most marked. The larger nerves (tibial, peroneal, crural, sciatic) showed changes similar to those in the roots. "It is noteworthy that in one and the same preparation thick swollen nerve fibers lay directly beside normal fibers or, on the other hand, beside fibers with simple or irregular medullary atrophy or fibers showing very marked nuclear proliferation in the sheath of Schwann, so that we have here the closest analogy with the state of the muscles." In like degree the degeneration was found in the muscular twigs of both forearms and could be followed with diminishing intensity into median, ulnar, and radial nerves in the upper arm. In the cutaneous nerves of upper and lower extremities no alteration was to be found. There were no abnormalities in the cervical sympathetic chain or its ganglia.

These autopsies, which are as representative as any of the positive findings in the "pure" Charcot-Marie-Tooth type of atrophy, gave Hoffmann a basis for speculation, so that reasoning from the conclusions of His, Kölliker, and Vignal, who from their embryological researches concluded that the grade of development of the peripheral nerves is proportional to the distance from the nerve roots, he wondered whether the peculiar evolution of the clinical picture might not have its explanation in the relative susceptibility of the more distant nerves to various influences. Omerod's (20) (1884) and Donkin's (7) (1890) descriptions of cases developing after measles caused Gowers (11) in 1899, to suggest an hereditary influence called into action by various toxins—(Hoffmann suggested alcohol and syphilis), but the evidence of heredity is so overwhelming and the evidence of specific toxins so slight that it hardly seems necessary to invoke the aid of a toxin.

A great deal has been written regarding the cases which do not fit the descriptions of Charcot-Marie and Tooth. They comprise many varieties, though they are relatively few in number. The chief basis,

in point of number, for subdivision or separation is the presence of palpable nerve trunks in the extremities. This group may be placed under the heading of progressive neural muscular atrophy since the individuals share to a greater or lesser extent a similarity in both the clinical and pathological picture. There are some, including more recently De Bruyn and Stern (6) and Russell and Garland (21), who hold there is a basis for considering it a separate entity. It was described as a "new" disease—"Hypertrophic Progressive Interstitial Neuritis of Childhood"—by Déjerine and Sottas (4) in 1893. A similar case had previously been described by Gombault and Mallet (10), in 1889, as "A Case of Juvenile Tabes." The summary of the case of Déjerine and Sottas is as follows:

> "In résumé we see that the case which we report, and on which we have performed an autopsy, constitutes a 'new' affection of the peripheral nerves—ascending neural interstitial hypertrophy prolonged into the posterior cord. Clinically, the affection presents the following characters:
>
> "It begins in early or late childhood with muscular atrophy of the extremities with alterations of sensibility, burning pains, kyphoscoliosis, hypertrophy and hardening of the nerve trunks, absence of cutaneous trophic disturbances. Then appear symptoms of motor incoördination with Romberg's sign, myosis with delayed or absent reaction to light (A-R pupils) and dynamic nystagmus."

In 1906 two cases were reported by Déjerine and Thomas (5). In the same year, before the Societé de Neurologie, Pierre Marie showed several cases and proposed, on clinical grounds, a separate subdivision from the Déjerine-Sottas group. Boveri (1) in 1911 added clarity to the subdivision but Hoffmann's (15) article, in 1912, is again an outstanding contribution. The sub-groups in this major division are seen to be three:

I. Type of Gombault-Déjerine-Sotta. Begins in early or late childhood with bilateral pes varus. Later there is marked ascending atrophy of the muscles of the four extremities. Ataxia. Outspoken disturbances of sensibility. Lightning pains, or on the other hand little pain of any sort. Argyll-Robertson pupils. [This sign is questioned by De Bruyn and Stern (6).] Positive Romberg. Myosis. Dynamic nystagmus. Loss of tendon reflexes. Fibrillary twitching. Kyphoscoliosis. Alteration in electrical conductivity with or without the reactions of degeneration.

Pathologically, one finds hypertrophy of the sheath of Schwann in the peripheral nerves with extension of the enlargement to the posterior roots and involvement of the spinal ganglia. The cranial and sympathetic nerves are also enlarged. There is degeneration in the posterior columns but only slight anterior horn involvement.

II. Type of Pierre Marie. Little muscle atrophy in the arms and hands. Hands hollowed because of tendon contracture. Disturbance of superficial and deep sensibility. Difficulty in gait and in maintaining an erect attitude from childhood on. Pes equino-varus. Atrophy of muscles of hands and feet not important, often slight. Kyphoscoliosis to greater or lesser extent. No lancinating pain, fixed pupils, ataxia or positive Romberg. There is, however, scanning speech, intention tremors, and exophthalmos. Pathologically, there is found enormous thickening in the peripheral nerves but no hypertrophy of the spinal roots, cauda equina, or cranial nerves. The spinal ganglia are thinner than normal. There is degeneration in the posterior columns, especially that of Goll, and also in the pyramidal and spinocerebellar tracts.

III. Type of Hoffmann. Begins with frequent turning-in of the ankle leading to bilateral equino-varus. Age of onset of 5 cases one, three, nine, fifteen, thirty to forty. Few fibrillary twitchings. No vasomotor changes. Small muscles of the hands atrophied, but good function preserved in the muscles of forearm, upper arm and trunk. Many rheumatic pains. Some paroxysms of tearing burning pain. Hyposensitivity to touch pain, pain, temperature, and impairment of position sense. Marked hypo-irritability of muscles to galvanic and faradic stimulation. The calf muscles show no trophic changes, nor alterations in function. The peroneal muscles are chiefly involved and the anterior tibials are next in order of pathological change. There are none of the symptoms of ataxia, scanning speech, etc., seen in the two groups preceding. No autopsies were reported by Hoffmann in his communication.

In a general way the intensity of the symptoms, though not of the muscular atrophy, varies directly with the degree of hyperplasia of the sheath of Schwann, which is the basic lesion in this group. There is little need to point out the resemblance of symptoms to those in tabes and Friedreich's ataxia in group I, and to multiple sclerosis in

group II. In this latter instance Hoffmann would draw a parallel between the abnormal proliferation of glia cells in multiple sclerosis and the cells of the sheath of Schwann in this group, both cell types being of ectodermal origin. Comparison has in similar fashion been made with von Recklinghausen's disease. Marie and Bertrand (18) and Souques and Bertrand (24) have studied the condition in detail histologically, and the latter are of the opinion that it is primarily "a lesion of the sheath of Schwann, accompanied ultimately by a degeneration of the myelin sheath and then the axis-cylinder. The lesions of the peripheral nerves, involving motor and sensory nerves simultaneously, react secondarily on the cord." The fact that the degree of hyperplasia of the cells of the sheath of Schwann does not determine the extent of muscular atrophy is easily seen by comparing Hoffmann's fifth case (1912) in this group and the case reported by Gierlich (9) (1909) of the "pure" Charcot-Marie-Tooth progressive neural muscular atrophy. This is further borne out in the autopsies recorded by Marinesco (1894) and Sainton (1899) in which the cases are classed generally with those of Virchow and Friedreich referred to above. In both instances the peripheral nerves were to all appearances normal, macroscopically. Microscopically, in the carefully studied case of Sainton (22), there is "marked thickening of the lamellar sheaths with notable interfascicular sclerosis" together with atrophy of the ganglion cells in addition to the alterations in the posterior columns. The pathological picture is well worked out and is thus quoted at length in discussions of progressive neural muscular atrophy. However, in this instance the atrophy began in the upper extremities in a man of forty. As will be seen, Gierlich's case is better suited for comparison with our patient B. C. and will be discussed below.

Less notable variants from the main group of progressive neural muscular atrophy are:

1. Those instances in which the atrophy begins in the lower extremities, the upper extremities remaining entirely unaffected (Sainton-Soca). Tooth's case (1889) may be classified in this group. 2. Atrophy limited to the upper extremities (Hänel).

Since the atrophy is limited, cases in these categories are sometimes referred to as *formes frustes*. They are discussed in the detailed comparative study of Dawidenkow (3) (1928) who, having placed all of

the cases of progressive neural muscular atrophy to be found in the literature into one great group—210 reports (124 males, 86 females), including 24 autopsies—and after comparing them carefully, as well as is possible under the circumstances, both clinically and pathologically, with no resulting common ground, makes the observation that the diversified cases have but one thing in common and that is heredity. Studied from the standpoint of heredity his conclusion is that the disturbance acts in all cases as a simple monohybrid dominant except for the Déjerine-Sottas group in which the characteristics of a recessive are seen.

As has been observed, the case here reported falls into the group as originally described by Charcot-Marie and Tooth. What is the pathological basis in this case? The peripheral nerves are not palpable. The atrophy is extreme. Those muscles which remain give no reaction of degeneration, which is a not unexpected finding in so long-standing an atrophy where function is retained by the surviving muscles. If one is to judge from the autopsies referred to (Virchow, Friedreich, Marinesco, Sainton), there is in this case either hypertrophy of the sheath of Schwann or increase in interstitial connective tissue of the peripheral nerves, or both, with degeneration in the posterior columns. Further light is thrown on the pathological picture by the autopsy on Gierlich's case (1909), which in its clinical aspect resembles more closely that of our patient B. C. This case is that of a boy who died at the age of seven, following bronchopneumonia. His disability also began at the age of two with atrophy of the leg muscles and subsequent atrophy of the muscles of the hands and the forearms several years later. His brother, one year older, was in the same condition. There were no disturbances of sensibility, or atrophy elsewhere. The pupils and coördination were normal. He had some weakness of the hands and arms. Microscopically, a careful study was made of the changes in the spinal cord. There was conspicuous atrophy of the cells in the dorsolateral columns, especially in the lower cord. Light areas (Weigert's stain) were found in two places, the first posteriorly in the lateral columns adjacent to the posterior horn, and the second in the posterior funiculus, affecting primarily the column of Goll. There were no changes in the anterior or posterior roots and the anterior horn cells were entirely normal. The peripheral nerves

(tibial and peroneal only examined) were thin and delicate macroscopically, but on section showed "degeneration and loss of numbers of nerve fibers; the myelin sheaths were swollen and pale though many had disappeared. Some of the axon-cylinders remaining show the reaction of degeneration. Intermingled among these there remain some entirely normal axons. There is thickening of the interstitial connective tissue."

The thickening of the interstitial connective tissue ("Bindegewebe") does not refer to the proliferating cells of the sheath of Schwann though that may have been meant. At any rate, as has been noted, the new growth of tissue, either of interstitial connective tissue or of the sheath of Schwann, at first affects but little the functional activity of the axons. Even in the late stages of atrophy a portion of the axons, both motor and sensory, function normally. Trophic changes may appear in the skin, though sometimes they are wholly lacking. In the case reported here they are very marked. In what way and through what structural and functional nervous mechanism this is brought about remains as yet to be answered.

SUMMARY

1. An advanced case of neural progressive muscular atrophy is herein described.

2. The original descriptions of this condition by Charcot and Marie and by Tooth are reviewed. The differential diagnosis is discussed, and the disease is contrasted with the other types of muscular atrophy.

3. A nephew, aged two years and nine months, presents changes in the skin of the feet said to be identical with those seen at the onset of the condition at the same age in this patient.

4. The etiology and pathological basis of this form of muscular atrophy is considered.

The writer wishes to acknowledge his indebtedness to Dr. Edward P. Carter for the privilege of reporting this case and for reviewing the manuscript.

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AN ARTICULATION IN A LARGE RENAL CALCULUS

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Interest was aroused by finding at autopsy a very large renal calculus moulded into the calyces of the kidney and into the beginning of the ureter, because it fell apart into two portions, precisely fitted to each other, like the surfaces of a joint.

This calculus was from the case of a white male, aged forty-five, who had been a patient in the Brady Urological Clinic, and who died November 3, 1928 (autopsy no. 10642). The clinical history, in so far as it relates to the point in question, shows that there had been a pain in the left flank in 1905, on account of which an operation was performed with the aim of fixing the kidney in place. Years later, in 1924, at a second operation, a stone measuring 2 by 1 cm. was removed. Four years later, the left kidney, at autopsy, measured 18.5 by 10 by 8 cm. The surface was studded with groups of small abscesses. The renal pelvis was greatly dilated. On section a huge, white calculus was found filling the lower two-thirds of the pelvis and projecting by a number of processes into the calyces (figs. 1 and 2). The upper extremity presented numerous papilla-like projections. The most interesting feature, however, was the fact that a lower, smaller portion was separate from the much larger upper portion, but that the two fitted together absolutely exactly, much like the articulation of two wrist bones. When pulled apart, the surfaces were found to be perfectly smooth, homogeneous in texture, polished, and in perfect apposition with each other at every point. The plane outlined by the articulation was not symmetrically dome-shaped, like a bowl, but had two main curves, nearly at right angles to each other, one approximately antero-posterior in its direction, and the other lateral. These curves are shown in outline in figure 3.

The larger fragment measured 7 by 6.5 by 6 cm. and weighed, when thoroughly dried, 180 grams. The smaller fragment measured 4 by 3.5 by 3 cm. and weighed, when dried, 32 grams. The combined weight

was, therefore, 212 grams. The cortex of the kidney presented numerous radiating abscesses. The pelvis was thickened, measuring 2 to 3 mm., and roughened and granular on its inner surface.

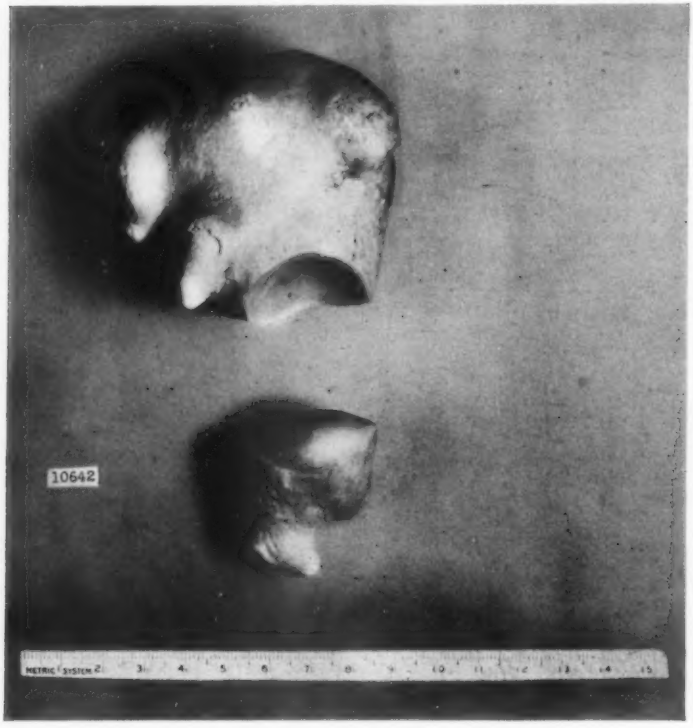

FIG. 1. HUGE RENAL CALCULUS

Note articular surfaces. Weight of larger fragment 180 grams; weight of smaller fragment 32 grams.

At the junction of the pelvis and ureter narrowing was so marked that a small probe was passed through it with difficulty. The ureter itself was dilated, measuring 2.1 cm. in circumference, and its wall was thickened.

Microscopical examination of the pelvis of this kidney showed no

intact epithelium. In its place was a lining tissue infiltrated with wandering cells. Outside of this was a broad, well defined band of smooth muscle. Next, there was a layer of connective tissue, in the

FIG. 2. ANTERIOR HALF OF LEFT KIDNEY, VIEWED FROM BEHIND, WITH CALCULUS IN SITU
Length of kidney 18.5 cm.

meshes of which were many wandering cells. Still further out was fat tissue.

The kidney showed an extensive disintegration of tubules with desquamation of the epithelial cells. Some of the glomeruli were dis-

tended with blood, while others were obliterated, but the majority were unaltered.

The tissue between the tubules was rich in wandering cells. Several abscess-like necrotic areas were present.

In brief, the picture was that of a comparatively inactive pyelonephritis with a dilated and thickened renal pelvis.

DISCUSSION

From a consideration of size alone, this calculus is unusual. It is said to be the largest one seen at the Brady Clinic. In comparison with giant calculi reported by others, however, it sets no record.

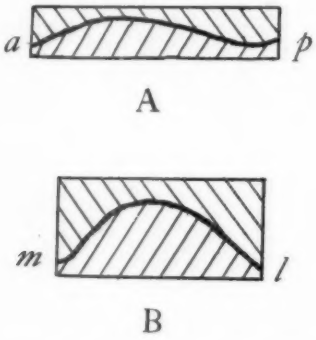

FIG. 3. (*a*) RECONSTRUCTION OF CURVE ALONG THE MIDDLE OF THE ANTEROPOSTERIOR PLANE OF THE ARTICULATION; (*b*) OF LATERAL PLANE
a, anterior; *p*, posterior; *m*, medial; *l*, lateral

Shield (1) in England, reported a renal stone which weighed 1 pound and half an ounce (468 grams), and measured 10 inches in circumference and $5\frac{1}{2}$ inches in its long diameter. He also referred to an enormous renal calculus in the musuem of St. Bartholomew's Hospital, a composite stalactitic mass on a central stem or core which weighed $36\frac{3}{4}$ ounces (1,042 grams), or over 2 pounds. Legueu, in a French encyclopedia of urology, records several instances of unusually large kidney stones. The largest weighed 2.5 kgm.

Of greater interest is the perfectly fitting articulation the line of which is hardly visible, since the contour of the whole is quite smooth.

It might occur to one that the two pieces had resulted from the fracture of an original single stone through some violent blow. In that

FIG. 4

FIG. 5

case perhaps only a slight friction of the fragments upon each other might have been required to smooth the apposed surfaces. But, as the photographs (figs. 4 and 5) show very clearly, there is an alternative

explanation which is better founded. It is quite evident that two calculi were formed of dark brownish material, doubtless urates, and that these were gradually enlarged by the deposit of laminae of a finer whitish material, probably largely composed of phosphates. Since even at death the whole mass filled only part of the pelvic cavity, we can not believe that the two calculi were held tightly together by the pelvic wall but were, even though they mould the calyces, allowed considerable freedom of movement. This may account for the deposit of laminae upon the opposed surfaces as well as upon those in contact with the pelvic wall and the upper surface over which lay the unoccupied space of the pelvis.

It is apparently to this freedom of movement that we may ascribe the precise adjustment of the adjacent surfaces of the two portions which have been ground and moulded together until their articulation is quite perfect. It is especially interesting to consider the complicated curves of this articulation. As described above and shown in the diagram and photographs of the sections, the curve is not that of a simple sphere. Instead, in one direction it is an arc of a small circle, in the other, at right angles, the arc of a much larger circle at the end of which the curve is reversed so that there is an overlapping lip. It is quite evident that this condition could not have been produced by a rotary grinding motion. Motion seems possible in one direction, that of the arc of the small circle, but very much less possible in the transverse direction, especially on account of the reversed curve of the overlapping lip. Possibly respiration and bending or twisting of the body played some part in the movement of these two stones against each other, but it seems probable that the rhythmic contraction of the renal pelvis which has been repeatedly observed in freshly excised hydronephrotic kidneys (2) was the most important factor, and in support of this view is the demonstration of the broad band of smooth muscle fibres in this renal pelvis.

Legueu, Fey and Palazzoli (3) studied the motility of the renal pelvis in freshly excised kidneys. Activity after suitable stimulus was found in the following conditions: tuberculous kidney, hydronephrosis, carcinoma, and calculus. It was not found when the renal pelvis and ureter were greatly sclerosed and thickened. In the only case of calculus, in which the stone was in the superior calyx, it was noted

both by pyeloscopy and after excision, that the region in contact with the calculus did not contract, but that all of the lower part of the pelvis contracted normally. In the present case, the above observation may have a bearing, as the calculus filled only about two-thirds of the renal pelvis.

It is of interest that, as pointed out by Dr. Rich, Schenckius, in 1608, pictures a renal calculus with an articulation almost exactly like the one described here (4).

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THE IMPORTANCE OF CIRCULATORY BALANCE IN THE SURVIVAL OF REPLANTED LIMBS[1]

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The experiment of replanting entire limbs without suture of vessels was first reported by Professor W. S. Halsted before the National Academy of Sciences in 1922 (1). This investigation was undertaken to "determine the amount and duration of the swelling which would result from the division of the vessels and lymphatics of a limb," and from these experiments Dr. Halsted hoped also to obtain proof of his contention that "permanent swelling of the arm following operations upon the axilla is probably invariably due to infection, an infection often so slight as to escape the observation of the surgeon." He termed this swelling of the arm, seen in some patients after the radical operation for cancer of the breast, *elephantiasis chirurgica* (2), and felt that it was unlikely that the excision of the axillary contents, lymphatics and veins, could alone be responsible for this swelling.

At first the experiments were directed toward the production of experimental elephantiasis, but during the eight years since Dr. Halsted's death the replantation method has been employed as a means of investigating the regeneration of lymphatics (3), experimental lymphoedema (4), and the importance of maintaining circulatory balance in a limb.

The original problem of producing a permanent experimental elephantiasis in the dog lacks one step in its completion, namely, the production of a lymphangitis in the skin of the replanted limbs (4).

The operation of replantation consisted of a complete circular division of all tissues in the mid-thigh, leaving in continuity only the denuded femoral artery and vein. The divided parts were carefully united by suture and primary healing ensued in practically all cases. Transient swelling of the replanted limb developed, but never gan-

[1] Presented before the National Academy of Sciences, September 18, 1930, Berkeley, California.

grene. Subsequent experiments showed that the operative procedure could be simplified by leaving the femur and the sciatic and femoral nerves uncut along with the two main denuded limb vessels. Thus all lymphatics and all blood vessels, except the single femoral artery and femoral vein to a limb had been divided. The postoperative swelling disappeared within seven or eight days, and at this time the replanted limb looked and was functioning like its control.

This type of replantation offered an ideal method for the study of the regeneration of lymphatics, arteries and veins under normal conditions (3) and also presented an opportunity to determine accurately the rôle of these vessels in maintaining adequate circulatory balance. It likewise permitted a careful experimental check on the dictum sactioned in the Great War (5) and investigated by Brooks (6), Holman (7), Theis (8), and others, *that when a large artery has been injured, the accompanying vein must be ligated to maintain adequate circulatory balance and to prevent gangrene.*

Our investigations showed that regeneration of the arteries across the replant line could readily be demonstrated roentgenographically on the third postoperative day and that a few vessels could be visualized as early as the end of the second day.

New venous anastomoses developed in the replanted area at the end of the fourth and the beginning of the fifth day, just when the swelling, as indicated by measurements, had reached a maximum, or was beginning to subside. Further proof of venous regeneration was shown by the fact that at this time ligation of the femoral vein caused no gangrene and did not produce further edema; in fact, this procedure caused a delay of only one or two days in the final subsidence of the swelling.

Definite lymphatic regeneration was demonstrated four days after operation at the time when the new venous channels were also being formed. These new lymphatic vessels occurred in the superficial group of lymphatics, and by the eighth postoperative day, when the edema had completely subsided, regeneration was physiologically adequate in both the deep and superficial sets of lymphatics. At this time no recurrence of the edema followed ligation of the femoral vein, but swelling reappeared when the lymphatics were blocked with granules of india ink. It was evident that the lymphatic system, by

its regeneration, was of importance in producing a subsidence of the postoperative edema, and that with the veins it formed a lymphaticovenous system, the function of which lay in disposing of the edema and in reestablishing the circulatory balance.

Concerning the importance of a circulatory balance the investigations of Professor Halsted on the method of curing arterio-venous fistula by quadruple ligations about the fistulous opening led him in 1920 (9) to state:

> "We are compelled, I believe, to subscribe to the view that some degree of equilibrium of the arterial and venous systems must be maintained . . . . granting this, there vanished any difficulty that there may have been in accounting for the very high percentages of gangrene observed to follow ligation of the artery in cases of arteriovenous fistula. . . . ."

In Brooks' study (6) of the diminution of gangrene after ligation of a large artery when simultaneous occlusion of the accompanying vein was performed, he quotes a personal communication from Dr. Halsted to the effect that simultaneous ligation of the vein is probably the preferable procedure in certain instances in which it is necessary to occlude the artery, but that it would seem advisable to wait until ligation of the vein is clearly indicated.

This present report[2] deals with experimental studies of the comparative value of ligating the artery and vein, either simultaneously or, as Professor Halsted suggested, by delaying the venous ligation until clearly indicated. The replantation method again offered a satisfactory experimental means of testing these clinical observations. In connection with this study, an investigation was made as to the exact time after replantation that the femoral artery or the femoral vein or both could be ligated without resultant gangrene. Section of the nerves to the limb or the alternative of leaving them intact at the time of replantation was found to have no appreciable influence on the circulatory balance.

In 17 cases of replantation with ligation of the femoral vein the venous occlusion was performed at the time of replantation in 7 ex-

[2] The earlier portions of this investigation were carried on in the Hunterian Laboratory of Experimental Surgery, Johns Hopkins Medical School, with the assistance of Dr. C. Y. Bidgood.

periments, and in the remainder at intervals of two, four, five, six, seven, nine, and eleven days after replantation.

When the venous occlusion was performed at the time of replantation, all of the animals quickly developed gangrene, became listless and appeared toxic; all died within forty-eight hours. Their blood pressure fell rapidly and some had generalized convulsions. These findings were difficult to explain since by ligating the femoral vein at the time of replantation the only means of a return flow of blood from the limb had been occluded.

Post-mortem examination revealed no hemorrhage or infection in the wound. However, in the tissues of the replanted limb as well as proximal to the replant line, extending in some instances into the tissues of the abdominal wall, large amounts of serous yellowish fluid, at times jelly-like, were found infiltrating the subcutaneous and muscular layers. The muscles of the replanted limb were swollen, lustreless and did not contract. Intravenous injections of small amounts of this yellowish exudate into a normal dog caused convulsions. Further evidence that this exudate was toxic was shown in an experiment in which the amputation of the mid-thigh was performed and the femoral vein ligated, but the tissues were not approximated and therefore the limb was not replanted. Gangrene developed, the exudate appeared in the amputated limb, but the dog was not toxic and had lived for more than two days at the time it was sacrificed.

Ligation of the femoral vein forty-eight hours after replantation led to gangrene but not to the death of the animals. They were quiet but took food. Apparently at this time the tissue spaces were not as patent in the operative area, and absorption in the proximal side of the wound was diminished.

Of two dogs in which the femoral vein was ligated four days after replantation, one did not develop gangrene. Ligations on the fifth, sixth, seventh, ninth and eleventh days were not followed by gangrene.

No gangrene developed, therefore, when the femoral vein was ligated four or more days after replantation.

Frequent thermal readings of the room and of the toes in the normal and replanted limbs were made on a number of these animals before and after venous ligation and offered a criterion of the imminence of gangrene at the crucial period four to five days after replantation.

When the color response of the skin and the thermal recovery of the operated limb to a temperature above that of the room appeared within four to five hours after ligation, survival generally followed. When the room temperature was warm (23 to 26°C.) the response was quicker than when it was cool (20 to 23°C.). Survival of the limb was rare when the animals were kept in a cold room (13 to 18°C.) at this critical time.

The femoral artery alone was ligated in 16 replantation experiments. Ligations on the fifth, sixth, eighth, ninth, tenth and eleventh days were all followed by gangrene. When the ligation was made on the twelfth day the appearance of gangrene was delayed. Of 3 ligations performed on the thirteenth day delayed gangrene appeared in one animal and two did not develop gangrene. No gangrene occurred in replanted limbs with arterial ligations made on the fourteenth, fifteenth and seventeenth days.

Thus, fourteen days after replantation the femoral artery could be ligated without the development of gangrene.

Simultaneous ligation of the femoral artery and vein was performed on 19 replanted limbs. In one instance the double ligation was done at the time of replantation and promptly resulted in gangrene.

Delayed gangrene developed in two cases in which the femoral artery and vein were ligated four days after replantation. Ligations performed on the fifth day following replantation in one instance did not induce gangrene, but in five other animals delayed gangrene ensued. Ligations in four dogs on the sixth postoperative day led to delayed localized gangrene in three instances, with no gangrene in the fourth animal. On the seventh day one dog had localized delayed gangrene and another did not develop gangrene. Ligations of the femoral artery and vein on the eighth, ninth, fifteenth and seventeenth postoperative days led to no gangrene.

Thus after seven days or more following replantation the simultaneous ligation of the femoral artery and vein did not lead to gangrene.

In these 52 experiments the relative importance, measured chronologically, of the arterial and venous systems and their interdependence in maintaining circulatory balance was definitely established. Gangrene did not develop after replantation when the simultaneous ligation of artery and vein was made seven to eight days later, whereas

ligation of the artery alone could not be safely performed until the fourteenth postoperative day. The ability of the afferent or venous system to establish an adequate circulatory balance and thereby to insure the survival of isolated tissues (replanted limbs) is greater and the effect obtained earlier (four to five postoperative days) than that of the arterial system (thirteen to fourteen days after operation). Disturbance of the arterial or efferent system such as occurs after ligation of the main artery in a replanted limb can be compensated for by ligation of the main vein, producing an adequate vascular balance on the seventh postoperative day. Thus the time of the first adequate response of the arterial system can be nearly halved by simultaneous ligation of the vein.

Following the suggestion of Professor Halsted, further studies were made on the effect of a delayed occlusion of the femoral vein after arterial ligation. Choosing a time, such as the ninth day after replantation, when in previous observations the ligation of the artery alone had invariably produced gangrene, 7 experiments were made in which the femoral vein was ligated, nineteen, five, three and one-half, three, three, three and three hours after occlusion of the femoral artery.

When the vein was ligated nineteen hours after occlusion of the artery a transient beneficial effect in color and in the temperature of the limb was seen, but gangrene developed two days later. Ligation of the vein five hours after the arterial occlusion improved the thermal condition of the limb but did not prevent the development of localized gangrene within 36 hours. No gangrene resulted following ligation of the vein three and one-half, three and three hours after the arterial occlusion. In two other experiments, localized delayed gangrene followed ligation of the vein three hours after ligation of the artery, because of a disturbance imposed upon the circulatory balance as shown below. Observations of the color of the skin and thermal changes in these five animals indicated that threatening gangrene might be prevented by occlusion of the vein when the appearance of the replanted limb became reddish blue and when the temperature of the toes approached that of a cool room (20 to 23°C.). Clinically, we are advised to keep warm the limb threatened with gangrene and in these experiments the animals were kept constantly in a warm room (23 to 26°C.). In two instances delayed gangrene ensued. In one

of these subjecting the threatened limb to a cold room (10°C. for two hours) two days after the vascular ligations (vein ligated three hours after artery) precipitated localized gangrene. The cause of gangrene in the other animal, in which the vein was ligated three hours after the artery, was definitely attributable to a perivenitis and deep lymphatic blockage induced for another purpose. This instance again emphasized the importance of the lymphatic system as a factor in maintaining an adequate afferent circulation.

SUMMARY

The replantation experiment offered a satisfactory method of studying the relative importance of the vascular systems, (arterial, venous and lymphatic), and their interdependence in maintaining adequate circulatory balance.

After replantation, regeneration of the arteries was demonstrated on the second and third days, of the veins on the fourth and fifth days, and of the lymphatics as early as the fourth postoperative day.

Experience with wounds in the World War has definitely indicated that in arterial ligation the incidence of gangrene may be diminished by the simultaneous occlusion of the accompanying vein. By means of the experimental replantation of limbs the effect of simultaneous ligation of the main artery and vein to an extremity as compared with the effect of ligation of the artery or vein alone has been established chronologically.

Gangrene does not develop if the main vein is ligated five or more days after replantation, whereas the artery alone cannot be safely occluded until the fourteenth day.

The time interval essential to a safe arterial ligation can be diminished to seven days when a simultaneous ligation of artery and vein is performed.

It is significant that the survival of the extremity after arterial ligation depends on the lapse of only a few hours before occlusion of the accompanying vein.

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THE DERIVATION OF ENCAPSULATED FORMS FROM NON-ENCAPSULATED HEMOLYTIC STREPTOCOCCI

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Encapsulated hemolytic streptococci, which are found sporadically in human and animal infection, are ordinarily regarded as independent entities, entirely distinct from the non-encapsulated strains. This view has arisen as a result of the fact that no one has succeeded in demonstrating a definite relationship between the encapsulated and non-encapsulated forms. It is the purpose of this paper to show that well known types of non-encapsulated hemolytic streptococci can be dissociated *in vitro* into an encapsulated or third form, in addition to the forms ordinarily spoken of as "S" and "R," with a consequent enhancement of virulence for mice and an acquisition of certain unusual characteristics already noted at the outset of the present work (1).

It may be well to review here some of the terms used in modern bacteriology, such as "rough" and "smooth," "R" and "S," and "dissociation." In the early work on the subject it was found that the colonies of the typhoid group (2), and of pneumococci (3) could exhibit two morphologically distinct forms, the one, appearing under certain conditions, having a rough or granular surface in contradistinction to the smooth shiny colony of the ordinary cultures. It was furthermore found that the organisms comprising these "rough" colonies were relatively non-virulent, whereas the "smooth," under proper conditions, were relatively virulent. The organisms of the smooth colonies were designated as "S" organisms and those of the rough colonies as "R" organisms. It was then found possible to effect a change from smooth to rough and vice versa. This reversible change was spoken of as "dissociation." Later, other bacteria were dissociated and it was found that virulence and colony morphology were not necessarily correlated in the sense just described. So, regardless

of the appearance of the surface of the colony, the letter "R" came to be used, instead of "rough," to imply relative non-virulence, and the letter "S" instead of "smooth" to indicate relative virulence. More recent work, however, has still further altered the usage of the terms R and S.

It has been shown that the S, relatively virulent, organisms in general differ from the R in that they possess type-specific substance. This was pointed out especially by Avery (4) for the pneumococcus, by Lancefield (5) for the hemolytic streptococcus, and by Julianelle (6) for Friedländer's bacillus. This type-specific substance seems to be a carbohydrate present in the capsule in the case of the S pneumococcus and S Friedländer bacillus, and a protein in the case of the non-encapsulated S hemolytic streptococcus. The R forms of pneumococci and Friedländer's bacilli are non-encapsulated and lack this type-specific carbohydrate substance. On the other hand, R hemolytic streptococci, which do not differ morphologically from the non-encapsulated S organisms, lack their type-specific protein substance. In the last analysis, the lack of this substance differentiates the R from the S forms. We must think of R organisms now as differing from S organisms more particularly on account of their lack of type-specific substance than because they are less virulent, for S organisms may be practically avirulent and still possess type-specific substance. This point is made very clear by Todd and Lancefield (7) in a paper dealing with hemolytic streptococci.

As the presence or absence of type-specific substance is the more fundamental differential point between R and S organisms, since morphology and virulence are not always evident or reliable criteria for this differentiation, we may review some of the properties of this ingredient of the bacterial body. One important aspect is the behavior of the type specific substance as an antigen. An anti-serum prepared against an organism possessing type-specific substance contains a type-specific antibody which will agglutinate that particular type of organisms only and will precipitate the type-specific substance of such an organism.

In the case of the pneumococci, for example, the S form of each different type contains a different type-specific substance and each is antigenically distinct from the other. Contrasted with this is the

species-specificity of R pneumococci derived from the various S forms by dissociation. These lack the type-specific carbohydrates, but possess a protein common to all pneumococci. Antisera prepared from the R forms of all types of pneumococci, therefore, cross-agglutinate. Despite the fact that this species-specific protein is contained in every pneumococcus, whether R or S, an anti-R-pneumococcus serum does not agglutinate an S culture. This, apparently, is because the capsule of the S form prevents contact between the species-specific substance and its antibody; for when the capsule is removed, an anti-R serum will then agglutinate the decapsulated S organisms. This is further borne out by the fact that in the case of the S hemolytic streptococci, where no capsule exists to prevent the contact and interaction between species-specific substance and its antibody, an anti-R streptococcus serum will agglutinate the homologous S form. In the case of the non-encapsulated streptococci, therefore, agglutination cannot be relied upon to distinguish between S and R. In order to demonstrate the presence of type-specific substance the precipitation reaction must be used.

The lack of type-specific substance in R organisms detracts from the efficiency of an antiserum prepared by them, and the active immunity set up by R is not as strong as that induced by S. This is not merely the effect of the relatively low virulence of the R forms, because in the case of the hemolytic streptococci it will be found that, although the S organisms of a particular strain may be made to vary greatly as to virulence, their type-specific substance may be constant and their anti-bacterial sera of equal potency.

When dissociating hemolytic streptococci the purity of R—its total lack of type-specific substance—is based on the precipitation reaction, because, as has been said, one cannot always be sure of the character of the organisms from the morphological appearance of the colonies. One of the reasons for the difficulty in determining the character of the organisms from gross inspection of the colonies, in the case of the hemolytic streptococci, is that there is not an abrupt reversion or change from S to a strictly pure R, and it is the intermediate variants or phases which constitute the indistinguishable colony forms. It is of importance to study the antigenic effects of these intermediate vari-

ants, for in the case of the organisms used for animal immunization by workers in vaccine and antitoxin laboratories, for example, unobserved variations in the cultures used may possibly be responsible for the well known fluctuations in the potency of different lots of any particular antiserum.

METHOD OF DISSOCIATION IN VITRO

Todd and Lancefield (7, 8) have made a careful study of the dissociation of non-encapsulated forms of hemolytic streptococci. They have shown that on a specially prepared chocolate agar, R and S colonies can be readily differentiated. However, in contrast to the appearance of pneumococcal colonies, the S colonies of hemolytic streptococci have a *matt* or dull and lusterless surface, while the R colonies are smooth and shiny. The former, therefore, they speak of as "matt" instead of "S," and the latter as "glossy." In any given strain the matt organisms are subdivided into matt "virulent" and matt "attenuated." Both of these have the same antigenic potentialities and only differ in their virulence because of the way they have been treated. Given a typical S or matt of low virulence, its virulence can be enhanced by mouse passage and decreased by leaving it in plain broth for two or three days. This potentiality to become more virulent is one differential point between attenuated S organisms and the stable avirulent R form.

As stated at the beginning of this paper, the purpose of the present work is to show that this separation of hemolytic streptococci into S and R, or matt and glossy, does not complete the story of their dissociation. Each hemolytic streptococcus which we have studied so far—including three scarlet fever, one erysipelas, one β-hemolytic streptococcus from a case of septicemia, as well as two of guinea-pig and rabbit origin—has exhibited the potentiality to dissociate into a well-encapsulated form, extremely virulent for mice. This highly interesting and unexpected fact came to light as a result of a study of the nature of the atypical or intermediate colony forms which appear under certain cultural conditions. If a typical S culture of a scarlet fever strain of hemolytic streptococcus, the well known New York 5, for example, is plated out on the meat infusion ascitic fluid

agar used in this laboratory,[1] the colonies will be found to be all of the same type, appearing as small droplets, with a dense grayish-white opacity, fading slightly at the margin. The surfaces are smooth and shiny (fig. 1).

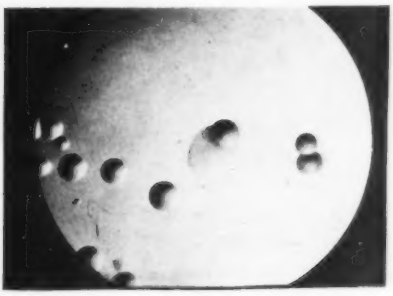

FIG. 1

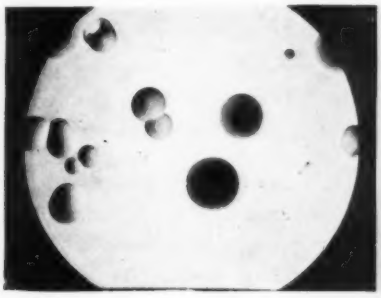

FIG. 2

FIG. 1. ORDINARY S HEMOLYTIC STREPTOCOCCUS COLONIES WITH ONE BUDDING TRANSPARENT PROCESS

N.B. These photographs are all of the same magnification. The colonies are on ascitic fluid agar.

FIG. 2. ORDINARY S HEMOLYTIC STREPTOCOCCUS COLONIES WITH TWO RIMMED COLONY VARIANTS

Dissociation to the encapsulated form was accomplished *in vitro* by subculturing every forty-eight hours in 2-per-cent blood or serum broth, or even by using isotonic meat infusion broth at a pH of 6.5. After subculturing for three or four weeks in this way, a few large,

[1] 820 grams chopped beef.
1640 cc. H_2O. Ice-box over night.
Bring to boil.
Filter through cheesecloth, cotton, and paper to clear.
Volume 1600 cc.
Add 32 grams Fairchild's peptone (2.0 per cent).
Add 8 grams NaCl (0.5 per cent).
Filter and adjust to pH 7.9 with N/1 NaOH. Boil one minute and check pH.
Filter through paper, and add 2 per cent agar.
Autoclave 15 pounds for ten minutes—pH 7.8.
Add 10 per cent ascitic fluid when ready to pour the plates.

opaque colonies appeared, the diameter of which was about twice that the original ones (fig. 4). These large colonies were often somewhat oval. At twenty-four hours their edges were regular, but at forty-eight hours they might be fluted. These large colonies were fished into blood broth and subcultured every forty-eight hours as before. In another week or two a third type of colony was found on streaking upon ascitic agar. These resembled "S" colonies surrounded by a well defined, narrow, transparent border, the surface of which was smooth and shiny (figs. 2 and 3). If india ink were used to demonstrate capsules, both the large, less opaque colonies and those with the transparent rims

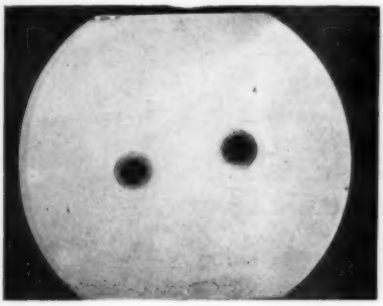

FIG. 3

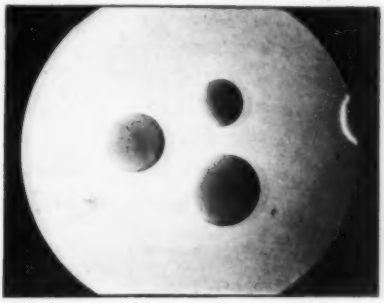

FIG. 4

FIG. 3. TWO RIMMED HEMOLYTIC STREPTOCOCCUS COLONY VARIANTS

FIG. 4. THREE OF THE LARGE "LESS OPAQUE" HEMOLYTIC STREPTOCOCCUS COLONY VARIANTS

were found to contain many encapsulated organisms (fig. 6). Further fishing of the rimmed variety of colony and subculturing in blood broth yielded more and more of these two atypical colony types, so that after about three months the streaked plate was made up mainly of these two types. Then it was noticed that two or three of the large, less opaque colonies, or colonies morphologically similar to S, had budding, transparent, smooth, shiny processes (fig. 1) which could be easily fished and on restreaking took the form of large, translucent, smooth, shiny colonies of irregular ameboid shapes and patterns. A few more subcultures and choosing of colonies free from any opacity shortly yielded the encapsulated form in its purity (fig. 5), the differ-

ence between this and the preceding stage merely being one of increased transparency.

It is interesting that although the scarlet fever strain New York 5 requires the above manipulation in order to effect dissociation to the encapsulated form, the scarlet fever strain C203, when plated out on the above medium, shows at the first plating the large and rimmed

FIG. 5. ENCAPSULATED HEMOLYTIC STREPTOCOCCUS COLONIES

FIG. 6

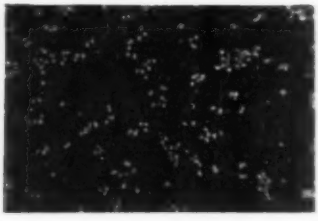

FIG. 7

FIG. 6. ENCAPSULATED HEMOLYTIC STREPTOCOCCUS DERIVED FROM THE NON-ENCAPSULATED HEMOLYTIC STREPTOCOCCUS SHOWN IN FIGURE 7

FIG. 7. NON-ENCAPSULATED HEMOLYTIC STREPTOCOCCUS

variants containing encapsulated organisms. It may be remarked that both these strains form only the matt type of colony on Todd's chocolate agar medium, as made by him (8).

Besides the distinctive colony morphology of the encapsulated organisms, their growth is homogeneous in broth, in contradistinction to the usual granular growth of the ordinary S. The capsules are

large (fig. 6) and have been retained even when subcultured repeatedly in plain broth over a period of three years. The organisms are not soluble in bile and their sugar fermentations do not differ from that of the non-encapsulated S forms. An anti-R or anti-S serum does not agglutinate them, but if the capsules are removed[2] the bacterial bodies may then be agglutinated by these antisera.

DISCUSSION

The ordinary forms of hemolytic streptococci are generally thought of as having no power to become encapsulated, and when encapsulated forms are encountered, the strain is always considered to be an entity and not a variant of the ordinary S or matt form. This conception, however, is not an accurate one, for we have dissociated several well known strains of non-encapsulated hemolytic streptococci to encapsulated forms *in vitro*—in fact, everyone of the seven strains that we have tried up to the present. It is of importance to attempt to derive encapsulated forms from all other hemolytic streptococci in order to determine whether encapsulation is a potential characteristic of the entire group, as these experiments seem strongly to indicate.

Before dissociation to the encapsulated form some of the strains were rather highly virulent for mice, some of very low virulence, but after dissociation to the encapsulated form each was of such a virulence that 0.25 cc. of a sixteen-hour plain broth culture diluted 100,000,000 times killed mice in from one to four days. This virulence has been maintained without animal passage in each case ever since dissociation was accomplished (one to three years). The organisms have also retained their capsules and colony morphology. It is clear, therefore, that a relatively non-virulent, non-encapsulated streptococcus can be dissociated *in vitro* to an exceedingly virulent encapsulated one, and also that when dissociated the tendency is to maintain *in vitro* this newly acquired form rather than to revert to a less virulent one. Occasionally and inconstantly a few colonies have been found, on plating the stock encapsulated cultures, which proved to be the R or glossy forms, but never the S or matt.

It is noteworthy that anti-S and anti-R sera fail to agglutinate the

[2] The capsules may be removed by suspending in 0.4 per cent NaCl solution and heating in a 56°C. waterbath for two hours.

encapsulated organisms, but do agglutinate them when the capsules are removed.

It is also interesting that the non-encapsulated S organisms in an agar focus in a rabbit tend to dissociate rapidly to the encapsulated form It is conceivable that this change to the encapsulated type may have a bearing on the production of anti-scarlet fever serum as prepared by the Dochez agar focus method. Studies of the toxin-producing power of the encapsulated forms and the chemical nature of the capsule are not yet completed.

CONCLUSIONS

1. Seven strains of non-encapsulated hemolytic streptococci, including erysipelas and scarlet fever strains, have been dissociated, *in vitro*, to encapsulated forms. It seems probable that the potentiality to form capsules may be a characteristic of hemolytic streptococci in general.

2. Dissociation to the encapsulated form enhances the culture's virulence for mice to a high degree.

3. The encapsulated forms have retained their morphology and virulence, without animal passage (*in vitro*) since their dissociation—a period which has been from one to three years.

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ON THE EXPERIMENTAL PRODUCTION OF SUPERFETATION[1,2]

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In an experimental study of ovulation in the rabbit we have found that it is possible to produce ovulation during gestation by injecting intravenously anterior lobe extract, or human urine of pregnancy or its concentrate. During the first five days after mating it is necessary to give relatively large doses of these active principles to induce ovulation, but a certain number of rabbits invariably ovulate a second time. We have repeatedly seen two sets of ova, which we have recovered by washing the tubes and uterus. The tubal set is unfertilized, the uterine set, of the ovulation which followed mating, is encountered as developing blastocysts. The occurrence of a second set of ova in these animals, in the presence of a previous, fertilized set, has led us to attempt to fertilize the second set of ova to see whether superfetation is experimentally possible.

Repeated attempts to fertilize the second set of ova failed. We have, however, succeeded recently in achieving our purpose, so that two sets of fertile ova have been recovered from tubes and uterus, respectively. This experiment seems to us of so much theoretical value that we believe it is worth reporting.

The protocol of this experiment is as follows:

On June 12 a healthy, mature female rabbit was mated at 4:50 p.m.

On June 16 at 12:15 p.m. this animal received 8 cc. of anterior lobe extract intravenously.

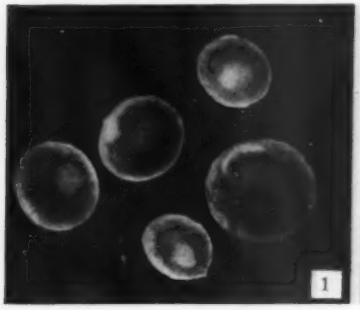
On the same day, at 4:30 p.m., we inseminated the animal artificially, by introducing about 2 cc. of fresh spermatic fluid into the vagina. The spermatic fluid was obtained by means of a glass pipette from the vagina of a second female which was allowed to have coitus.

[1] This research was aided by a grant from the Linton Fund.

[2] Submitted for publication June 25, 1931.

On June 17, at 9:15 p.m., the animal was killed and autopsied. Tubes and uterine cornua were washed for ova. The ovaries showed corpora lutea of two separate ovulations, one set distinctly larger than the other.

On washing the uterine cornua, two normal, developing blastocysts were obtained on the right side, three normal blastocysts on the left side. On washing the tubes, one segmenting and one unsegmented ovum were found on the right side; five segmenting ova were found on the left side. The segmenting ova were apparently normal, showing segmentation stages of from 3 to 5 cells (figs. 1 and 2).



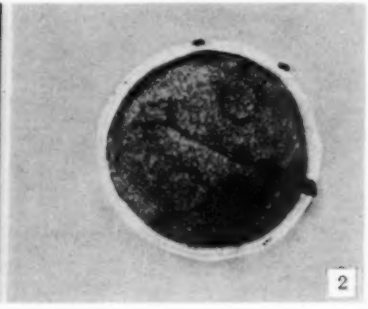


FIG. 1. PHOTOGRAPH OF NORMAL, DEVELOPING BLASTOCYSTS, AFTER FIXATION IN BOUIN'S FLUID, OBTAINED BY WASHING THE UTERINE CORNUA. × 12

FIG. 2. PHOTOGRAPH OF ONE OF THE SEGMENTING OVA, OBTAINED BY WASHING THE TUBES, AFTER FIXATION IN BOUIN'S FLUID AND CLEARING. × 300

Mention should be made of the anterior lobe extract. The product used was that prepared by Dr. Bugbee and was sent to us by Dr. Kamm, of Parke, Davis & Company.

The observation here recorded demonstrates the possibility of successfully fertilizing a second set of ova in the presence of living blastocysts. It shows that the blastocysts have not prevented a second set of ova from becoming fertilized. Nor have the changes occurring supposedly in ovaries, tubes and uterus, after fertilization of the first set, prevented a second set of normal ova from being discharged and becoming fertilized. Moreover, the sperm has reached

the second set of ova without having been deleteriously affected by either blastocysts or the changes in the mucosal lining of the uterus and tubes following the initial mating. That the second set of ova were not fertilized by sperm surviving from the insemination of mating seems established by a series of our own observations, as well as from the findings of Hammond and Asdell (*Brit. Jour. Exper. Biol.*, 1926, vol. 4, p. 155). The latter showed that spermatozoa retain their fertility in the female reproductive tract only up to 30 hours. Our own evidence is based on our failure to obtain two sets of fertilized ova from two ovulations, but with only a single insemination.

The present experiment fails to support the concept of the primacy of the ovum. The ova do not apparently exert an inhibitory influence upon ovulation, fertilization and maturation of a second set of ova. That there is some kind of strong inhibition, however, to ovulation during pregnancy is shown by the natural disinclination of the animal to mate during pregnancy or pseudo-pregnancy, by the fact that in a few instances in which mating during pregnancy has been observed by us, ovulation has not resulted, and thirdly, by the fact that it requires, as we have observed in a series of experiments, relatively large doses of urine extract to overcome the refractory condition of the ovary to a second ovulation.

This demonstration of the possibility of superfetation opens a number of avenues of investigation. It will be interesting to determine the fate of the second set of fertilized ova in the presence of the initial set of blastocysts. Will the period of transit through the tubes become altered? Will the spacing of the first set of ova or the reaction of the endometrium following their implantation prevent successful implantation of the second set? Does the time of implantation of the ovum depend upon the stage of development of the ovum or upon the condition of the endometrium? These and other questions can be answered by further similar experiments. By this method of controlling the arrival of a second set of ova in the uterus we may be able to investigate the factors in the endometrium which are necessary for successful implantation.

FURTHER OBSERVATIONS UPON THE EXPERIMENTAL PRODUCTION OF OVULATION IN THE RABBIT[1,2]

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From the Departments of Anatomy and Obstetrics, Johns Hopkins University

The present paper is a report upon further experimental investigations upon ovulation in the rabbit. A previous communication (1931) dealt with the effect of the injection of urine from pregnant animals upon ovulation, and the experiments that are now being reported represent a continuation of our studies. The data presented are from various experiments designed to study in further detail the physiological properties of the substance in human urine of pregnancy that induces ovulation in the rabbit and, furthermore, to test the conditions under which ovulation may occur.

MATERIAL AND METHODS

Healthy, mature, isolated rabbits have been used. As controls for our experiments enough human urines of known pregnancy have been employed to be certain of the sensitiveness of our rabbit stock.

As in the previous experiments (Snyder and Wislocki, 1931) frank ovulation has been made the sole criterion of a positive reaction in mature rabbits. Our reasons for disregarding partial reactions, such as blood follicles, have been given in our previous paper. The question of blood follicles will, however, be considered again in the sections of this paper dealing with the reactions of the ovaries in immature, pregnant and pseudo-pregnant rabbits. The criteria for identifying ovulation (recently ruptured follicles) need not be stated again.

The special technique followed, in so far as it is necessary to give it, will be presented under the respective series of experiments.

Many of our experiments have been carried out with the aid of a so-called "luteinizing" hormone prepared by Parke, Davis and Com-

[1] This research was aided by a grant from the Linton Fund.

[2] Submitted for publication July 10, 1931.

pany. This preparation, according to a communication from Dr. E. A Sharp of this company, is obtained by concentrating human urine of pregnancy. The first allotment of this extract which we used was stated to contain about 100 rat units per cubic centimeter; three subsequent allotments, with which the majority of our experiments were carried out, contained about 50 rat units per cubic centimeter. This substance will be referred to, in the presentation of our results, as concentrated urine of pregnancy. The term "luteinizing hormone" implies a too specific action of the preparation, besides failing to make clear the actual nature of the substance which we are using.

A number of our experiments, paralleling those performed with the active extract of human urine, have been carried out with the aid of anterior lobe extract of the hypophysis (beef) prepared by Dr. Bugbee of Parke, Davis and Company. This preparation, stated to contain the growth hormone of the anterior lobe, has a powerful effect upon the ovaries of rabbits.

OBSERVATIONS

As a matter of convenience our observations will be presented and discussed under the following headings:

1. The recovery of an active substance in rabbit's urine following the injection of concentrated human urine of pregnancy.
2. The disappearance of an active substance, causing ovulation, from human urine following hysterectomy.
3. The occurrence in various fluids (amniotic fluid) and tissues (anterior lobe) of a substance causing ovulation in the rabbit.
4. Differences in intravenous, intraperitoneal and subcutaneous routes of administration of concentrated urine of pregnancy.
5. On the changes produced in the ovaries of young rabbits by injection of concentrated human urine of pregnancy and anterior lobe extracts.
6. Ovulation in the pregnant rabbit induced by intravenous administration of concentrated urine of pregnancy or anterior lobe extract.
7. Ovulation produced experimentally in the rabbit compared with similar experiments in the cat.

The recovery of an active substance in rabbit's urine following the injection of concentrated human urine of pregnancy. Concentrated

human urine of pregnancy (P., D. & Co.) (1 cc. = 50 rat units) produces ovulation in mature, isolated rabbits. We have not attempted to determine the minimal effective dose, but 0.1 cc., injected intravenously, has given positive results in several instances. It seemed of considerable interest to us, from the standpoint of determining the nature of the principle of human urine of pregnancy to see whether the active substance, causing ovulation, could be recovered from the urine after injection into a rabbit. For this purpose we injected 10 cc. of concentrated human urine of pregnancy into an ear-vein of a rabbit. Urine collected from this rabbit during the following eight hours and injected into another mature rabbit did not make the second rabbit ovulate. In a second experiment, in which a rabbit was given 20 cc. of

TABLE 1

The recovery of an active substance in rabbit's urine following the injection of concentrated human urine of pregnancy

	AMOUNT OF CONCENTRATED HUMAN URINE INJECTED INTO 1ST RABBIT	AMOUNT OF URINE FROM 1ST RABBIT INJECTED INTO 2ND RABBIT	EFFECT ON OVULATION IN 2ND RABBIT
Expt. 1.....	10 cc. concentrated urine (P., D. & Co.)	10 cc. intravenously	No ovulation
Expt. 2.....	10 cc. concentrated urine (P., D. & Co.)	10 cc. intravenously	No ovulation
Expt. 3.....	20 cc. concentrated urine (P., D. & Co.)	10 cc. intravenously	Ovulation

concentrated human urine, the active substance was excreted by the kidneys since the second rabbit, which received rabbit's urine from the first, ovulated. These experiments are presented in table 1.

It appears from these observations that with sufficiently large dosage, the principle in human urine of pregnancy that causes ovulation is excreted by the rabbit's kidneys. These findings suggest that the reason why an active principle similar to the human one is not found in pregnant rabbit's urine is not because of the failure of the rabbit's kidneys to eliminate such a substance.

The disappearance of the active substance, causing ovulation, from human urine following hysterectomy. These observations throw some light upon the disappearance from human urine of the substance which produces ovulation, after the product of conception has been removed.

In the present cases, the termination of pregnancy at an early stage has been necessitated by complications, such as nephritis.

The relatively short interval required for the total disappearance of the substance which produces ovulation in the rabbit is apparent. It is surprising, if the substance is of hypophyseal origin, that it should disappear so soon after hysterectomy.

Here might also be mentioned a human urine from a case of abdominal pregnancy. Ten cubic centimeters of this urine, obtained on the

TABLE 2

The disappearance of the active substance, causing ovulation, from human urine following hysterectomy

DESCRIPTION OF HYSTERECTOMY	NUMBER OF DAYS AFTER OPERATION	RESULT
Case 1. Hysterectomy at 2 mos. (Taylor)....	1. Specimen obtained 2 days after operation	Ovulation
	2. Specimen obtained 4 days after operation	Ovulation
	3. Specimen obtained 5 days after operation	No ovulation
	4. Specimen obtained 6 days after operation	No ovulation
Case 2. Hysterectomy at 4 mos. (Bass).....	1. Specimen obtained 2 days after operation	Ovulation
	2. Specimen obtained 3 days after operation	No ovulation
	3. Specimen obtained 4 days after operation	No ovulation
Case 3. Hysterectomy at 3½ mos. (Beach)....	1. Specimen obtained 4 days after operation	Ovulation
	2. Specimen obtained 5 days after operation	No ovulation
	3. Specimen obtained 6 days after operation	No ovulation
Case 4. Hysterectomy at 8 mos. (Bzozowski)..	1. Specimen obtained 3 days after operation	Ovulation

day of hysterectomy for abdominal pregnancy, produced ovulation in a rabbit.

The occurrence in various fluids (amniotic fluid) and tissues (anterior lobe) of a substance causing ovulation in the rabbit. A series of experiments has been undertaken to test a number of substances which might be expected to produced ovulation. The substances used and the results obtained from this series are shown in table 3.

It will be observed that amniotic fluid from the human produces ovulation, whereas aminotic fluid from the sow is negative. This is in keeping with the observation that the urines of pregnant animals do

not cause ovulation in the rabbit. Amniotin (Squibb), which is prepared from amniotic fluid of the cow, is negative, as was to be expected.

Anterior lobe extracts give positive results in the rabbit. Acid as well as alkaline extracts of anterior lobe, supplied by Dr. E. M. K.

TABLE 3

Effects of various substances, besides human urine of pregnancy, upon ovulation in the rabbit

SUBSTANCE	NUMBER OF TESTS	SOURCE	METHOD OF ADMINISTRATION	OVULATION
Amniotic fluid	1	Human, 4th month	10 cc. intravenously	Positive
	1	Human, term	10 cc. intravenously	Positive
	1	Sow, 75 mm. fetuses	10 cc. intravenously	Negative
	1	Sow, 123 mm. fetuses	10 cc. intravenously	Negative
Amniotin (Squibb)	2	Cow	2 cc. intravenously	Negative
Anterior lobe (P., D. & Co.)	7	Beef	1–3 cc. intravenously	Positive
Anterior lobe (Geiling)	3	Beef	4 cc. intravenously	Positive
Cerebrospinal fluid	2	Human, term	7 cc. intravenously	Negative
Adrenal cortex extract (Swingle and Pfiffner)	3		0.5 cc. (2) intravenously 3 cc. (1) intravenously	Negative
Placental extract (Geiling)	1	Human, term	4 cc. intravenously	Negative
Placental transplant	1	Human, term	Subcutaneous implantation	Negative
Chorion	1	Sow, 175 mm. fetuses	Subcutaneous implantation	Negative
Endometrium (from pregnancy	1	Sow, 175 mm. fetuses	Subcutaneous implantation	Negative

Geiling, produced ovulation. Anterior lobe extract prepared by Parke, Davis and Company, stated to contain the growth hormone, invariably causes mature rabbits to ovulate. Doses of from 1 to 3 cc. are effective. We have not attempted to ascertain the minimal effec-

tive dose of this substance. Wolfe (1930) has reported the interesting observation that the hypophysis of the sow undergoes cyclical physiological changes in that at different stages of the cycle the amount of anterior lobe substance necessary to induce ovulation in the rabbit varies.

Cerebrospinal fluid from pregnant women proves to be negative in two instances in the rabbit. Similarly, according to Zondek (1931), cerebrospinal fluid of pregnancy does not affect the ovary of the mouse. However, Aronowitsch (1930) obtained a certain number of positive results on ovaries of mice.

Adrenal cortex extract prepared by Swingle and Pfiffner does not produce ovulation in the rabbit.

The several placental materials, whether human or animal, which we have tried, do not induce ovulation in the rabbit. Philipp (1930) reports that human placenta implanted in the rabbit produces blood points and luteinization of the interstitial tissue of the ovary. He points out the similarity of the substance in the placenta that affects the ovary, to anterior lobe hormone. Our experiments give no evidence that ovulation follows administration of placental substance. Our observations are, however, too few to permit any far-reaching conclusion. Of two extracts which we have used, one from the hypophysis of cattle, the other from the human placenta, both prepared by the same method by Dr. Geiling, the hypophyseal extract produced ovulation, the placental extract did not. This observation suggests that there is a difference in these two tissues in the occurrence of the substance causing ovulation.

Differences in intravenous, intraperitoneal and subcutaneous routes of administration of concentrated human urine of pregnancy. Friedman (1929) reports that intraperitoneal injection of urine from pregnant women produces luteinization of the resulting corpora hemorrhagica in the rabbit ovary. On the other hand, a single intravenous injection of urine from pregnant women produces ovulation in the rabbit. In a later paper (1930) he says of these observations, "Clearly, then, the mode of administration and the dosage must be considered as factors which are possibly responsible for variations in the response of the ovaries to the injection of any material."

In concentrated human urine of pregnancy (P., D. & Co.) we have a

convenient substance for testing the validity of Friedman's statement. We have carried out two sets of experiments on groups of four rabbits. These indicate the rôles of the factor of dosage and of the route of administration in reference to an active substance.

As in our previous experiments (1931), we have considered ovulation the most trustworthy criterion of a positive result. Given subcutaneously or intraperitoneally, 1 cc. doses of concentrated human urine of pregnancy do not produce ovulation; the same amount or a lesser amount given intravenously produces ovulation. If, however, the dosage is increased to 4 cc., ovulation occurs regardless

TABLE 4

Effect upon ovulation in the rabbit of concentrated human urine of pregnancy (P., D. & Co.) administered by different routes

ROUTE OF ADMINISTRATION	DOSAGE	RESULT
	cc.	
Expt. 1:		
Subcutaneously	1	No ovulation
Intraperitoneally	1	No ovulation
Intravenously	1	Ovulation
Intravenously	0.1	Ovulation
Expt. 2:		
Subcutaneously	4	Ovulation
Intraperitoneally	4	Ovulation
Intravenously	4	Ovulation
Intravenously	0.1	Ovulation

of whether the principle is given subcutaneously, intraperitoneally or intravenously.

These findings support the observation of Friedman that the results obtained by the injection of urine of pregnancy vary with the route of administration. They suggest, however, very strikingly the rôle which dosage plays in the results obtained.

On the changes produced in the ovaries of young rabbits by injection of concentrated human urine of pregnancy (P., D. & Co.), and of anterior lobe extract (P., D. & Co.). We have carried out a series of observations to determine the age at which ovulation can first be induced in the rabbit by a single injection of concentrated human urine of pregnancy

(P., D. & Co). These experiments have shown that before the age of twelve weeks, or approximately three months, no macroscopically visible change is produced in the ovaries by a single intravenous injection of as high as 2 cc. of concentrated urine. In rabbits of about twelve weeks of age, however, the ovaries commence to be markedly altered by such an injection. Doses of 2 cc. of concentrated urine produce an oedematous condition of the ovaries associated with the appearance of numerous large, fresh blood follicles, but only rarely a ruptured follicle. Seven healthy young rabbits, twelve to fourteen weeks old, all presented an identical picture of the ovaries, consisting of numerous large, bloody, follicles with only two normally ruptured follicles among them. Many of the blood follicles appear to be rather deeply embedded in the ovarian stroma, so that they do not protrude as markedly as is the case in blood follicles which sometimes occur in older rabbits. The stroma appears to be rather oedematous and often infiltrated with free blood. The dosage given these animals ranged from 2 to 5 cc. of concentrated urine administered intravenously.

Two rabbits of the same age (twelve weeks) given single doses of 2 cc. of anterior lobe extract (P., D. & Co.) presented a similar although not quite so intense a reaction. Two other rabbits given 4 cc. each of anterior lobe extract showed a complete identity in the resulting picture.

Thus, the first macroscopically visible response of the ovaries to two substances which produce ovulation in the mature rabbit is the formation of unruptured bloody follicles. This reaction is interesting, since it throws some light on the nature of blood follicles. It demonstrates that the ovary is, as a rule, incapable of reacting at this critical period by ovulation to the stimulus of the two substances under consideration. It accounts possibly for the fact that several observers report blood follicles, instead of frank ovulation, as occurring with great frequency following the injection of human urine of pregnancy. During the period of immaturity the formation of blood follicles is the normal response of the healthy animal to concentrated human urine of pregnancy and anterior lobe extract. On the other hand, by the age at least of seven months, ovulation has become the normal response. In normal, mature rabbits, however, we have observed blood follicles to occur with considerable regularity after the injection of concentrated

urine of pregnancy within the refractory period following a previous ovulation (in pregnancy and pseudo-pregnancy).

An observation concerning the minimal dosage necessary to evoke a

TABLE 5

Ovulation in the pregnant rabbit induced by intravenous administration of concentrated human urine of pregnancy (P., D. & Co.) or anterior lobe extract (P., D. & Co.)

NUMBER OF RABBIT	DAY OF PREGNANCY (NUMBER OF DAYS AFTER MATING)	AMOUNT OF OVULATION-PRODUCING SUBSTANCE INJECTED INTRAVENOUSLY	RESULT IN OVARIES
63	2 days 15 hrs.	10 cc. concentrated urine of pregnancy	Ovulation
92	2 days 15 hrs.	10 cc. concentrated urine of pregnancy	No ovulation
94	3 days 1 hr.	9 cc. concentrated urine of pregnancy	No ovulation
96	3 days 1 hr.	9 cc. concentrated urine of pregnancy	No ovulation
90	3 days 20 hrs.	8 cc. anterior lobe extract	Ovulation
95	4 days 3 hrs.	8 cc. concentrated urine of pregnancy	No ovulation
98	4 days 3 hrs.	8 cc. concentrated urine of pregnancy	No ovulation
50	4 days 2 hrs.	2 cc. concentrated urine of pregnancy	No ovulation
44	4 days 2 hrs.	2 cc. concentrated urine of pregnancy	No ovulation
74	5 days 14 hrs.	4 cc. concentrated urine of pregnancy	Ovulation
86	5 days 20 hrs.	3 cc. concentrated urine of pregnancy	Ovulation
82	5 days 20 hrs.	3 cc. concentrated urine of pregnancy	Ovulation
73	5 days 22 hrs.	4 cc. concentrated urine of pregnancy	No ovulation
81	6 days	4 cc. concentrated urine of pregnancy	Ovulation
47	6 days 14 hrs.	3 cc. concentrated urine of pregnancy	Ovulation
59	6 days 15 hrs.	4 cc. concentrated urine of pregnancy	Ovulation
49	7 days	2 cc. concentrated urine of pregnancy	Ovulation
48	9 days	2 cc. concentrated urine of pregnancy	Ovulation
52	11 days	10 cc. human urine of pregnancy	Ovulation
70	13 days	2 cc. concentrated urine of pregnancy	No ovulation
54	13 days	2 cc. concentrated urine of pregnancy	Ovulation
71	17 days	2 cc. concentrated urine of pregnancy	Ovulation
35	21 days	2 cc. anterior lobe extract	Ovulation
26	25 days (?)	10 cc. human urine of pregnancy	Ovulation
32	26 days	2 cc. concentrated urine of pregnancy	Ovulation
49	28 days	0.2 cc. anterior lobe extract	No ovulation
54	29 days	2 cc. concentrated urine of pregnancy	Ovulation
48	30 days	0.1 cc. concentrated urine of pregnancy	No ovulation
62	30 days	0.2 cc. concentrated urine of pregnancy	No ovulation

reaction in the immature ovary is of interest. Two rabbits, thirteen weeks of age, were taken. One was given intravenously 0.1 cc. of concentrated urine of pregnancy, the other received 0.2 cc. The ovaries of the former receiving 0.1 cc. showed no macroscopical reac-

tion, whereas the ovaries of the latter receiving 0.2 cc. exhibited a number of large blood follicles, but no recently ruptured follicles.

Ovulation in the pregnant rabbit induced by intravenous administration of concentrated human urine of pregnancy (P., D. & Co.), or of anterior lobe extract (P., D. & Co.). It is possible by the injection of concentrated human urine of pregnancy or of anterior lobe extract to produce ovulation in the pregnant rabbit. The results of these experiments are shown in table 5.

It can be seen that ovulation may be induced during gestation by intravenous administration of either concentrated urine of pregnancy or anterior lobe extract. It requires, however, relatively large doses, and the ovaries are almost refractory to ovulation, even with quite large doses of concentrated urine, during the first four days of pregnancy, the period immediately following the ovulation of mating. From the fifth day on, however, ovulation can be obtained by relatively large dosage with nearly uniform regularity. From this period on, a second set of corpora is obtainable, as a rule, by administering 2 to 4 cc. of the active substance. The threshold for ovulation in the pregnant rabbit, however, remains high contrasted with that for the non-gravid rabbit in which we have observed repeatedly that as little as 0.1 cc. of the concentrated urine of pregnancy suffices to produce ovulation.

That the second ovulation in these animals results in the formation of true corpora lutea and is not merely a process of luteinization of a set of follicles with retained ova has been established by washing the tubes and uterus for ova. In some eight animals which have been examined two sets of ova have been washed from tubes and uterus. In five other animals a set of ova has been recovered from the tubes in the presence of blastocysts, either free or implanted, in the uterus. Thus, we have evidence of true ovulation occurring in pregnancy. The production of a second set of ova in the presence of a preceding fertile set raises the interesting possibility of fertilizing the second set of ova—an initial step in the production of experimental superfetation. This we have accomplished in one animal (Wislocki and Snyder, 1931).

Of interest in connection with the difficulty of producing a second ovulation soon after the first is the observation that, in the non-gravid rabbit as well, a refractory period follows ovulation during which a

second ovulation cannot be induced. The period of complete refractoriness is, as in the gravid animal, approximately four days, but is subject to variation and dependent to a great extent upon dosage. Subsequently to this period, however, the non-gravid rabbit can be made to ovulate again with minimal dosage by the end of three weeks, whereas the gravid animals exhibit a higher threshold during the entire period of gestation. Another point of interest is the frequency with which ovulation can be induced. We have made one non-gravid rabbit ovulate by giving 2 cc. of concentrated human urine of pregnancy intravenously at 5, 8, 7 and 7-day intervals, so that at the end of four weeks the ovaries contained 5 sets of corpora lutea corresponding to the five injections. If the injections had been made at lesser intervals than this, repeated ovulations would not have occurred. From our various data we may conclude that there appears to be a refractory period during which a second set of follicles does not tend to rupture, but the length of this period depends, in our experience, upon the dosage of the active principle administered.

It is noteworthy that during the refractory period doses of concentrated urine of pregnancy which are insufficient to cause ovulation frequently produce blood follicles. This we have noticed particularly in our pregnant animals in which the ovaries were refractory to ovulation upon receiving large dosage during the first four days of pregnancy. It is interesting that the two groups of animals in which we have observed with regularity the appearance of blood follicles are (1) immature rabbits, and (2), pseudo-pregnant or pregnant animals immediately after a previous ovulation.

A further question of interest concerning these experiments is whether the injection of concentrated urine of pregnancy during pregnancy, or the resulting ovulation, alters noticeably the course of pregnancy by producing death or abortion of the fetuses. Most of our animals of this series have been killed in order to wash out the tubes and uterus, so as to obtain the ova. Several of the animals advanced in pregnancy have aborted or the fetuses have died and undergone absorption. This termination might well be attributed to the fact that subsequent to the injection of the luteinizing principle, a laparotomy had been performed under ether anaesthesia in order that the ovaries could be examined. The ether anaesthesia, as well as a cer-

tain amount of unavoidable handling of the uterus in order to admit examination of the ovaries, might well account for the termination of pregnancy. Two animals of the series, however, give excellent evidence, we believe, that the concentrated urine of pregnancy, or the second ovulation, does not necessarily produce abortion or death of the fetuses. Thus the uteri of animals nos. 49 and 48, receiving concentrated urine and ovulating on the seventh and ninth days of gestation,

TABLE 6

Effect upon the ovaries of cats of intravenous injections of human urine of pregnancy, concentrated urine of pregnancy and anterior lobe extract

CAT NUMBER	MATERIAL	RECIPIENT	RESULT
1	10 cc. human urine from 7 mos. pregnancy	Mature cat, not in heat	No ovulation
2	10 cc. human urine from 7 mos. pregnancy	Mature cat, not in heat	No ovulation
3	2 cc. concentrated urine of pregnancy	Mature cat, not in heat	No ovulation
4	2 cc. concentrated urine of pregnancy	Mature cat, not in heat	No ovulation
5	10 cc. human urine from 7 mos. pregnancy	Cat (early implantation)	No ovulation
6	2 cc. concentrated urine of pregnancy	Cat (early implantation)	No ovulation
7	2 cc. concentrated urine of pregnancy	Cat (late pregnancy)	Ovulation
8	3 cc. concentrated urine of pregnancy	Cat (late pregnancy)	No ovulation
9	4 cc. anterior lobe extract	Mature cat, not in heat	No ovulation
10	4 cc. anterior lobe extract	Cat (late pregnancy)	No ovulation

respectively, when opened 22 and 19 days later, contained living fetuses, almost at term.

A comparison of ovulation in the rabbit with ovulation in the cat. We have carried out a small series of experiments on the effect upon the ovaries of cats of intravenous injection of human urine of pregnancy, of concentrated urine of pregnancy (P., D. & Co.) and anterior lobe extract (P., D. & Co.) in order to compare this animal with the rabbit. The cat like the rabbit does not ovulate spontaneously. The results obtained upon cats differ from those obtained in rabbits.

It will be seen by consulting the table that, with one exception, none of the ten cats which we injected ovulated. The five cats that were not pregnant did not ovulate after the injection of ordinary human urine of pregnancy, with relatively large doses of concentrated urine, or after the injection of anterior lobe extract, while in our experience any healthy, mature, non-gravid rabbit would have ovulated with similar doses of the same substances. The five cats in question, were not in heat according to vaginal smears which were made. We did not extend our observations to oestrous cats in which ovulation would presumably have occurred.

Of three pregnant cats receiving concentrated human urine of pregnancy two failed to ovulate after doses which would have made rabbits of equivalent periods of gestation ovulate. Similarly, a pregnant cat given 10 cc. of human urine of pregnancy, and one given 4 cc. of anterior lobe extract, failed to ovulate under conditions in which rabbits would have ovulated.

The results in this small series of experiments is sufficiently clear-cut to permit the conclusion that the active principle contained in human urine of pregnancy behaves differently in the same range of dosage in cat and rabbit. Given in larger quantities in the cat it may well be that ovulation would be obtained with the same frequency as in the rabbit. If this should prove to be the case, it will illustrate, as has been emphasized in several sets of our experiments upon rabbits, the important rôle of dosage in the results obtained with the active principle of urine. In the guinea-pig also, Jarès (1931) has failed to induce ovulation by intravenous injection of urine of pregnancy. In the anoestrous ferret, Hill and Parkes (1930) have found that ovulation can be induced by large, daily injections of urine extract over a period of ten days. These results in no way compare with the ease with which ovulation can be induced in the rabbit. The experiments on guinea-pigs, on ferrets, and our own on cats, indicate that there is a difference between the mechanism for the release of ovulation in the rabbit as compared with the guinea-pig and cat.

SUMMARY

1. Following intravenous injection, the active substance of concentrated human urine of pregnancy that causes ovulation in the rabbit can be recovered from rabbit's urine.

2. The active substance causing ovulation in the rabbit disappears from human urine within several days following removal of the pregnant uterus.

3. An active substance causing ovulation in the rabbit has been found in human amniotic fluid and extracts of the anterior lobe of cattle. Negative findings have been obtained with human cerebrospinal fluid and human placental implants, as well as with various substances from other mammals.

4. Ovulation in the rabbit occurs after a smaller dose of concentrated human urine of pregnancy given intravenously than when given subcutaneously or intraperitoneally.

5. In immature rabbits, prior to the fourth month, a single intravenous injection of concentrated urine of pregnancy produces no visible macroscopic change in the ovaries. Beginning with the fourth month a striking change occurs in the ovaries following injection of concentrated urine or anterior lobe extract. This response in immature animals is characterizied by the occurrence of edema, hemorrhage and blood follicles.

6. In the pregnant rabbit, ovulation can be induced throughout pregnancy by the injection of concentrated human urine of pregnancy. There is a tendency towards inhibition of ovulation especially during the first four days of gestation.

7. In the cat, ovulation does not occur in the range of dosage of concentrated human urine which induces ovulation in the rabbit.

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